Direct Development in *Rostanga arbutus* (Angas) (Mollusca: Nudibranchia) and the effects of temperature and salinity on embryos reared in the laboratory

R.A. Rose [1]
School of Biological Sciences
University of Sydney
NSW 2006

[1] Present address: Western Australian Marine Research Laboratories
P.O.Box 20, North Beach, W.A. 6020

ABSTRACT

Rostanga arbutus from New South Wales is a small, strawberry-coloured, intertidal, dorid nudibranch which lays between 150 and 700 eggs per transparent egg ribbon. Zygotes are orange-red and approximately 184 μm in diameter. Development is direct (or ametamorphic Type 3 as defined by Bonar, 1978). Hatching occurs 15 days after oviposition at 20-21°C. Embryos pass through a reduced veliger stage during morphogenesis into benthic juveniles (approximately 234 μm in length). During the veliger stage, embryos cannot retract into their shells and they do not have an operculum, a functional velum with locomotory cilia or pedal glands. In embryos from different populations, the shell is either cap-like or type 1 (as defined by Thompson, 1961). Embryos produced by adults from Long Reef have a narrow range of tolerance over 20 temperature-salinity combinations (10, 15, 20, 25, 30°C and 20, 27, 34, 40‰), all of which are within the ranges that occur naturally in the field. Tolerance to temperature is widest (20 to 25°C) at 34‰ salinity. Rate of development to hatching increases with temperature over the range 20 to 25°C. Both hyposaline (20, 27‰) and hypersaline conditions (40‰) increase the percentage of abnormally developing embryos. If embryos are exposed, even briefly, to temperatures near 13 or 32°C in conjunction with salinities ranging from 27 to 40‰, at any time between oviposition and later stages of cleavage, they are unlikely to survive. These results correspond well with areas in which egg masses are found in the lower and middle reaches of the shore during summer and autumn (December to May), where conditions are less extreme than higher up the shore.

INTRODUCTION

Studies on the development of nudibranchs have been numerous (for reviews see Thompson, 1967 and Bonar, 1978), but few have dealt with the combined effects of temperature and salinity on direct-developing embryos or larvae. Research that has been published deals mainly with planktotrophic and lecithotrophic species (e.g., Dehnel & Kong, 1979 and Harris *et al.*, 1980).

Rostanga arbutus (Angas 1864) is a direct-developing, strawberry-coloured, dorid nudibranch (15 mm or less in length) which occurs on the eastern and western coasts of Australia (Thompson, 1975). At Long Reef, New South Wales, adults are found intertidally throughout most of the year, but their egg masses are restricted to mid-and lower-shore rock pools and are found only during summer and autumn (December to May) (personal observations). Both adults and embryos during daylight low tides are exposed to fluctuations in temperature and salinity which annually range from 13 to 37°C and 28 to 38‰, respectively.

Although development in *Rostanga pulchra* and *R. muscula* has been described as planktotrophic (Chia & Koss, 1978 and Rose, 1985, respectively), there is no detailed account of a direct-developing species of *Rostanga*. This paper describes the development of *R. arbutus* and the combined effects of temperature and salinity on the rate of development, proportion of embryos reaching various embryonic stages and proportion of embryos at various stages developing normally. This information contributes towards a better understanding of the reproductive ecology of direct-developing opisthobranchs.

MATERIALS & METHODS

Animals and egg masses were collected from rocky intertidal shores at Shelly Beach (Port Macquarie), Long Reef (Sydney) and Callala Point (Jervis Bay), New South Wales during spring, summer and autumn of 1977 to 1979. Specimens brought back to the laboratory from Long Reef were used for the temperature and salinity experiments. Only one egg mass per adult was used so that the results could be related to environmental rather than genetic factors. Temperatures and salinities tested were arbitrarily chosen but spanned the annual range of conditions found at various collection sites during daylight low tides. Environmental cabinets were used to obtain the desired incubation temperatures. Subdued lighting on a 12 h light/12 h dark cycle was provided in each cabinet. Hyposaline media were prepared by adding distilled water to filtered seawater and hypersaline media by evaporating filtered seawater. Media were changed daily, each medium first being brought to the appropriate temperature. The pH of all media fell between 7.1 and 8.2.

The embryonic stages reached and the proportion of normal embryos were recorded for groups of 50 randomly selected embryos in each treatment, at time intervals based on the developmental rate of embryos incubated at 20-21°C and 33-35‰ salinity. Pieces of egg masses immersed in the appropriate medium were incubated in 10 ml vials with perforated plastic lids or in 50 ml glass bowls, depending on their length. Preliminary experiments indicated that cutting egg masses into shorter pieces had no effect on development. Observations on development were made with a phase contrast microscope. Photographs were taken with a camera and flash unit attached to the microscope.

Two types of experiments were performed:

Constant temperature-salinity experiments

To test the effects of temperatures and salinities kept constant throughout embryonic development, four salinities (20, 27, 34, 40‰) and five temperatures (10, 15, 20, 25, 30°C) were used, giving a total of 20 treatments. Each treatment contained a section of an egg mass with 70 to 280 zygotes which had been produced at 20-21°C and 33-35‰. Experiments were replicated twice and four to six egg masses were used per experiment. Embryonic stages used as a scale for measuring the developmental progress up to hatching were those most easily recognised and are listed in Table 1.

To compare the effects of different constant temperatures and salinities on the rate of development, methods of Patel & Crisp (1960), Walne (1965) and Lough & Gonor (1971) were used

to estimate the "standard" developmental times of different embryonic stages. These times were obtained from embryos reared at 20-21°C and 33-35‰.

In figures 1 and 2, the position of each embryonic stage on the ordinate axis represents the standard developmental time required for embryos to reach that stage, when expressed as a proportion of the mean total developmental time up to hatching. Unless otherwise indicated, an embryonic stage was considered to be reached when a mean of 50% or more of the embryos was at or beyond that stage.

Effects of constant temperatures and salinities on the proportions of embryos which had reached or passed various embryonic stages at specific times were analysed with either a Student's t-test or analysis of variance (ANOVA). All proportions were transformed to arcsines before each analysis to stabilise variances. Significant differences between temperature-salinity treatments detected by the analyses of variance were interpreted with Student-Newman-Keuls multiple range tests (SNK, $P \geq 0.05$; Sokal & Rholf, 1969).

Varied temperature-salinity experiments

These experiments considered the effects of transferring embryos at four different stages of development from an optimal temperature-salinity regime (23°C/34‰S) to six non-optimal regimes (17°C/27‰S, 17°C/34‰S,17°C/40‰S, 32°C/27‰S, 32°C/34‰S, 32°C/40‰S) and vice versa. In some cases both temperature and salinity were changed, in others only temperature was altered, leaving the salinity at 34‰S. Zygotes reared at 23°C in 27,34 and 40‰S (three treatments) throughout their embryonic development were used as controls. This gave 12 treatments plus three controls for both types of transfer experiments. Each type of transfer and control experiment was replicated three times and the number of embryos per egg mass section was approximately the same as in experiments in which conditions were constant.

The four embryonic stages tested were easily identifiable and were characterised by different developmental processes. They were: fourth cleavage to morula (4C-M); gastrula (G); early veliger (EV); and juvenile (J). Zygotes that had not yet expelled the first or second polar body were used for transfer experiments in which embryos were initially kept at non-optimal regimes before being placed in the optimal one.

RESULTS

Development

Over a period of several days after copulation, *Rostanga arbutus* produces one or two type A or a^1 egg-mass ribbons (as defined by Hurst, 1967, or Fernandez-Ovies, 1981, respectively) (Plate 1, A). These transparent egg masses are spirally arranged and contain between 150 and 700 reddish-orange eggs which had been fertilised internally before oviposition. Each zygote is encased in a double-membraned capsule (Plate 1, B). The mean diameter ± s.d. of ten zygotes is 184 ± 8 μm.

Embryonic development up to hatching takes 15 days at 20-21°C. Chronology of the main developmental stages is presented in Table 1, and some of these stages are illustrated in Plate 1 (B to J).

The general pattern of cleavage, gastrulation and embryogenesis is similar to that described for other dorid nudibranchs (e.g., Thompson, 1958 and Rose, 1983). Briefly, cleavage beyond the two cell stage is spiral and at the third cleavage, formation of the first quartet of micromeres is asynchronous and dexitropic (Plate 1, C). Gastrulation is by a mixture of epiboly and invagination, giving the embryos a heart-shaped appearance (Plate 1, D). During the trochophore stage invagination and eversion of the shell gland begins directly opposite the stomodoeum.

Embryos at the early veliger stage have a prominent, rudimentary velum and metapodium, and a thin, delicate shell which is two-thirds complete. The velum is cloaked in fine cilia and the shell gland can often be seen at this time as a mound of clear cells on the antero-dorsal surface of the embryo. The stomodoeum can be seen as a shallow depression on the antero-ventral surface. Anal cells appear on the postero-ventral surface during early veliger, just to the right of the midline (Plate 1, E). By late veliger they will have migrated to their definitive position (the future site of the proctodeal invagination), on the right side of the embryo.

At the middle veliger stage, embryos have a completely formed shell and perivisceral cavity and can be seen slowly rotating inside their capsules. The metapodium is now a thick, rod-shaped structure without an operculum, and its ventral surface is covered with fine cilia. The velar rudiment is bilobed and its thick marginal cells are covered with short, fine cilia. The alimentary tract is partially differentiated at this time.

During middle veliger, a clear spherical vesicle forms on the right dorsal side of the embryo at the same time as the hindgut is being formed. This vesicle is possibly the secondary larval kidney as found in *Hoplodoris nodulosa* (Rose 1983) and in *Philine denticula* (Horikoshi, cited by Bonar, 1978). Alternatively, it may be homologous with the "oval glassy vesicle" described for the direct-developing embryos of *Casella obsoleta* (Soliman & Gohar, 1967). In fully developed embryos of *Rostanga pulchra*, a similar clear vesicle is labelled as the midgut diverticulum by Chia and Koss (1978). Towards the end of middle veliger, the mantle fold thickens around the anterior margin of the shell aperture. Embryos at this time begin developing a faint eye spot at the base of the velum on the right side.

During the late veliger stage, embryos have two eye spots, a fully developed alimentary tract, propodium, metapodium (without an operculum) and two large, partially transparent velar lobes with thick margins covered with short cilia (Plate 1, F and G). Towards the end of this stage, as the first signs of the juvenile form are beginning to appear, the embryos develop a heart and two small statocysts. The heart, situated postero-dorsal to the statocysts, is light-coloured, oblong and beats irregularly. The statocysts, which are embedded in the basal tissue of the foot, disappear shortly before hatching. As in other direct-developing species (Thompson, 1967), pedal glands do not develop.

Morphogenesis of the juvenile form begins about halfway through the late veliger stage on the 12th day after oviposition and occurs gradually over 24 h. During this period several morphogenetic events occur concomitantly. By the time the heart and statocysts have developed, the mantle fold has thickened and reflexed over the dorsal and lateral surfaces of the shell as well as underneath it. While the mantle fold spreads over the shell posteriorly, postules or papillae form on its dorsal surface (Plate 1, F and G). Underneath the shell, mantle tissue continues to thicken and slowly proliferate backwards, well behind the advancing edge of the mantle tissue on the outside surface of the shell. The thickening mantle tissue underneath the shell appears to fuse with the perivisceral membrane and this action is probably responsible for lifting the shell and pushing it off the visceral mass. Meanwhile the velum is resorbed and the foot broadens and extends backwards along the ventral side of the embryo. The anal cells have disappeared by this time.

The shell is gradually cast when the mantle tissue has covered four-fifths of it. Shells are either cap-shaped (Plate 1, F) or type 1 (Plate 1, H) as defined by Thompson (1961). Type 1 shells are similar to those of the veligers of *R. pulchra* (Chia & Koss, 1978) and *R. muscula* (Rose, 1985). The shape of the shells of *R. arbutus* appears to depend on where the parents of the embryos come from; adults from Long Reef produce embryos with cap-shaped shells, while those from Shelly Beach, and Callala Point produce embryos with type 1 shells.

By the time the shells have been jettisoned, embryos have taken on the juvenile form and can be seen rolled-up and revolving inside their capsules. At the juvenile stage, the dorsal epidermis develops a mantle skirt which appears to originate from the reflexed mantle tissue covering the dorsal region of the visceral mass. As in *Adalaria proxima* (Thompson, 1958) and *Tritonia hombergi* (Thompson, 1962), the mantle tissue of *R. arbutus* now spreads anteriorly, encircling the rhinophoral rudiments and fusing together at the front of the head. Monaxon spicules develop in the lateral margins of the mantle skirt and in the dorsal papillae.

Just before the capsules rupture on the 15th day, the membranes become thin and increase in area. Newly hatched *R. arbutus* juveniles resemble those of *R. pulchra* (Chia & Koss, 1978). Their viscera are in a detorted state and they range in length from 218 to 250 μm (Plate 1, H and I). Unfed juveniles 20 days after hatching are approximately 570 μm long.

Constant temperature-salinity experiments

Out of 20 regimes tested, hatching occurred only in 34 and 40‰S at 20 and 25°C. Regardless of salinity, embryos incubated at 10,15 and 30°C failed to develop beyond the early stages of cleavage or blastula. Embryos reared in 34‰S, hatched four days sooner at 25°C than at 20°C (Fig.1). Embryos reared in 40‰S also developed faster at 25°C than at 20°C, but not beyond the MV stage (Fig. 2). After this stage, very few embryos (3% or less) developed and those that did hatched at the same time at both temperatures. Embryos reared in 40‰S at 20 and 25°C not only developed more slowly than those reared in 34‰S at these same temperatures but they were abnormal at hatching (Plate 1, J). This suggests that the upper limit of tolerance for normal development in *R. arbutus* is between 34 and 40‰S.

The total percentage of embryos which reached or passed various embryonic stages and the percentage of this total which were abnormal for different treatments are illustrated in Figure 3. The proportions of embryos cultured in the three greater salinities (27, 34, 40‰S) at 15, 20, 25 and 30°C (12 treatments), which reached or passed the 1C stage at the 12th h after oviposition, were not significantly affected by temperature, but they were affected by salinity and by the interaction of salinity and temperature (2-factor ANOVA, Table 2). At each of the four temperatures tested, the mean percentage of embryos reaching or passing 1C was largest in 34‰S, second in 40‰S, third in 27‰S and least in 20‰S (SNK Test). The effect of interaction of temperature and salinity on the proportion of embryos reaching the 1C stage at hour 12 was due to 30°C/40‰S and 15°C/27‰S; under these treatments the proportions were lower than any of those from the other ten treatments analysed.

Development up to or beyond G stage on day 4 occurred in 27,34 and 40‰S at 20 and 25°C and in 34‰S at 15°C. Only 1% of the embryos from 15°C/34‰S regime reached or passed this stage on day 4. The proportions of embryos from three greater salinities at 20 and 25°C (six treatments) which were at, or beyond, G at this time were significantly affected by salinity and its interaction with temperature but not by temperature (Table 2). At both of these temperatures, the mean proportion of embryos at G was significantly lower at 27‰S than at 34 and 40‰S. The effect of interaction of temperature and salinity on the proportion of embryos developing was due to the following four treatments: 20°C/34‰S; 20°C/40‰S; 25°C/34‰S and 25°C/40‰S. Under these treatments the mean percentage reaching stage G on day 4 was greater than those at 20°C/27‰S and 25°C/40‰S but not significantly different from each other (SNK test).

Development of embryos up to or beyond the EV stage on day 5 occurred only in 34 and 40‰S at 20 and 25°C. The proportions of embryos from these four treatments which were at, or beyond, EV at this time were significantly affected by temperature and salinity (Table 2). Regardless of these two salinities, more embryos developed beyond the EV stage at 25°C than at 20°C.

Embryos reared in 34‰S at 20 and 25°C were the only ones to reach hatching on day 16, and the proportion of embryos which had hatched at this time was not significantly different at the two temperatures (t-test, t'= 1.0, 1df; $P \geq 0.05$). Embryos cultured in 40‰S at 20 and 25°C hatched on day 18, however, as stated before, less than 3% hatched at either temperature.

Varied temperature-salinity experiments

The results of the control experiments indicate that at 23°C, embryos cultured in 27‰ S failed to hatch while 99% and 2% of those cultured in 34 and 40‰S, respectively, hatched on the 12th day after oviposition (Table 3). Embryos exposed to the three experimental salinities (27, 34, 40‰S) at 32°C for as little as 12 hours after oviposition failed to develop when transferred to optimal conditions of 23°C/34‰S. When embryos were transferred from the optimal condition to any of the salinities at 32°C, they also failed to develop and hatch.

Embryos reared at 13°C failed to develop beyond the 4C-M stage at 27 and 40‰S and beyond the G stage at 34‰S. If embryos which were initially reared in 34 and 40‰S at 13°C were transferred at the 4C-M stage to the optimal conditions, they took two days longer to hatch than control embryos. Moreover, 18% or less of the embryos hatched on the 14th day (Table 4), and all of them were abnormal (Plate 1, J). If embryos were transferred from the optimal conditions to 13°C at various developmental stages, those kept at 27‰S failed to hatch while less than 5% of the embryos at 34‰S hatched and then only when transferred at the 4C-M stage (Table 5). All embryos

which hatched after being transferred at the 4C-M stage to 13°C were abnormal. Less than 5% of embryos kept in 40‰S at 13°C hatched, regardless of the stage at which they are transferred. At 13°C development was prolonged for at least a month (Table 4).

DISCUSSION

Development

Bonar (1978) separated direct development (Type 3 as defined by Thompson 1967) into two categories, depending on the degree of developmental condensation observed during morphogenesis. In one of the categories, capsular metamorphic development (meta. Type 3), the embryos pass through a veliger stage within the egg capsule before metamorphosing into benthic juveniles and hatching. During the veliger stage the embryos have "larval" structures which would be functional if they were artificially released into the plankton (Bonar, 1978). The other category, ametamorphic development (ameta. Type 3) contains those embryos which have a reduced or completely suppressed veliger stage and as a consequence do not progress through any recognizable metamorphosis. Instead, the embryos gradually develop into a benthic juvenile. As examples of ametamorphic development, Bonar (1978) cities *Cadlina laevis* (Thompson, 1967) and *Casella obsoleta* (Gohar & Soliman, 1967). However, these species differ from each other in that in *Casella obsoleta* the veliger structures are not nearly as reduced as in *C. laevis.* In many respects the veliger structures of *Casella obsoleta* are similar to those of *R. arbutus,* except for two major differences. First, the visceral mass of the embryos of *Casella obsoleta* never differentiates into an alimentary tract with a perivisceral cavity. Second, *Casella obsoleta* does not develop a larval shell, just a thin delicate cap which covers the posterior end (Gohar & Soliman, 1967).

R. arbutus embryos at the veliger stage have several reduced and atrophied larval structures (i.e. a reduced larval shell in some embryos and no operculum, pedal glands, or locomotory cilia on the velum). If these embryos were liberated at the veliger stage, they would not be able to survive in the plankton. In keeping with Bonar's (1978) scheme of developmental patterns for species with direct (non-pelagic) development, *R. arbutus* should be classified as having capsular ametamorphic development.

Tardy (1970) presented a classification scheme for nudibranchs which divided them into two major types on the basis of the shape of the protoconch. Species with type 1 protoconch (Thompson, 1961) are categorized as having planktotrophic development (Type 1; Thompson 1967), while those with a type 2 protoconch (Thompson 1961) have lecithotrophic development (Type 2; Thompson, 1967). In species with Type 1 development, the dorsal epidermis originates from an eversion or reflexion of the mantle fold, which spreads posteriorly and laterally over the visceral mass during metamorphosis. In species with Type 2 development, it originates from the floor of the mantle cavity. According to this classification, *R. arbutus* has Type 1 development (Plate 1, G) and formation of its dorsal epidermis is similar to that found in *Adalaria proxima* (Thompson, 1958) and *Tritonia hombergi* (Thompson, 1962), *Aeolidiella alderi* (Tardy, 1970) and *Hoplodoris nodulosa* (Rose, 1983).

Formation of the dorsal epidermis in *R. arbutus* is reminiscent of that found in contemporary cypraeid gastropods (Thompson, 1962). These observations lend further support to the hypothesis that dorids evolved from shelled ancestors by progressive enclosure of the shell by the mantle (Thompson, 1962).

Torsion in *R. arbutus* is similar to that found in *T. hombergi* (Thompson, 1962), *Cuthona nana* (Rivest, 1978) and *H. nodulosa* (Rose, 1983). The only evidence of visceral rotation in *R. arbutus* is the short migration of the anal cells, at embryogenesis, from the postero-ventral surface of the embryo to the definitive larval position on the right lateral surface of the embryo. Otherwise all of the visceral organs appear in their post-torsional position. Torsion in *R. arbutus* is not like that found in the dorid *Adalaria proxima.* In *A. proxima* (Thompson, 1958), not even the migration of the anal cells is seen as a vestige of torsion; the viscera and anal cells are, at the time of their first appearance, in the definitive larval position.

Due to distorted viewing encountered with encapsulated embryos of *R. arbutus* during morphogenesis into the juvenile, the exact process of detorsion cannot be observed. The

appearance of prematurely hatched juveniles, however, suggest that detorsion is completed only after the shell is jettisoned. Premature juveniles can be seen with their clear vesicle and anal complex postero-laterally, just to the right of the midline of the body. As they mature, the anal complex and clear vesicle move to their definitive position, along the midline at the posterior end of the body.

Effects of temperature and salinity on development

The results of experiments using constant temperature and salinity indicate that the range of temperature tolerance of *R. arbutus* is greatest at the optimal salinity tested (34‰S). This is a common phenomenon among marine organisms (Kinne, 1964). Over a range of 5°C at 34‰S, embryos of this species show a poikilothermal response to temperatures compatible with normal development (i.e, a decrease in hatching time with increasing temperature). Although this response occurs over a narrow temperature range, the results agree with those for other gastropods (Ganaros, 1958; Scheltema, 1967; Spight, 1975) and opisthobranchs (Hadfield and Switzer-Dunlap, 1984).

Embryos *R. arbutus* from Long Reef are stenosaline and stenothermic in comparison to those of other opisthobranchs (e.g. Hagerman, 1970; Shyamasundari & Najbuddin, 1976; Harris *et al.*, 1980). At hypersaline conditions of 40‰S, the percentage of embryos hatching is reduced and the time to hatching is increased. A similar situation is found with the planktotrophic embryos of *Doto coronata* and *D. fragilis* when they are cultured at hyposaline conditions below 34‰S (Kress, 1975). At the highest temperature tested (30°C), the blastomeres of *R. arbutus* embryos, during early cleavage, lose their cohesive properties and segregate like that observed in the planktotrophic embryos of *Cadlina luteomarginata* (Dehnel & Kong, 1979).

Embryos which are placed in non-optimal conditions immediately after oviposition and are subsequently transferred to the optimal conditions at the 4C-M stage do not completely recover from the effects of the non-optimal conditions. More than 80% of them fail to develop to hatching and those that do are abnormal. These results support the hypothesis that if embryos of this species at Long Reef were to be exposed, even briefly, during the time from oviposition to later cleavage stages, to temperatures near 13 or 32°C in conjunction with salinities ranging from 27 to 40‰S they would be unlikely to survive. At Long Reef, embryos are likely to experience temperatures near 13°C in combination with the salinities tested at the end of the breeding season (late autumn). Embryos would rarely encounter temperatures near 32°C, especially in combination with the lower salinity tested, as egg masses are generally deposited in the lower and middle region of the shore (pers. obs.). A possible exception might occur if egg masses were deposited in upper-shore rock pools during summer after a low tide and heavy rainfall.

The relatively narrow range of temperatures and salinities tolerated by embryos of *R. arbutus* may explain why egg masses are laid in the lower and middle reaches of the shore at Long Reef during summer and autumn. In view of the wide geographic range of this species in Australia (Thompson, 1975), it would be interesting to determine whether or not embryos from populations at different latitudes and embryos from adults acclimated to different regimes show local adaptations to the same temperatures and salinities mentioned above. Moreover, cyclical temperature-salinity experiments (e.g. Christiansen & Costlow (1975) and Lucas & Costlow (1979)), would give a more realistic interpretation of the effects of environmental conditions on development.

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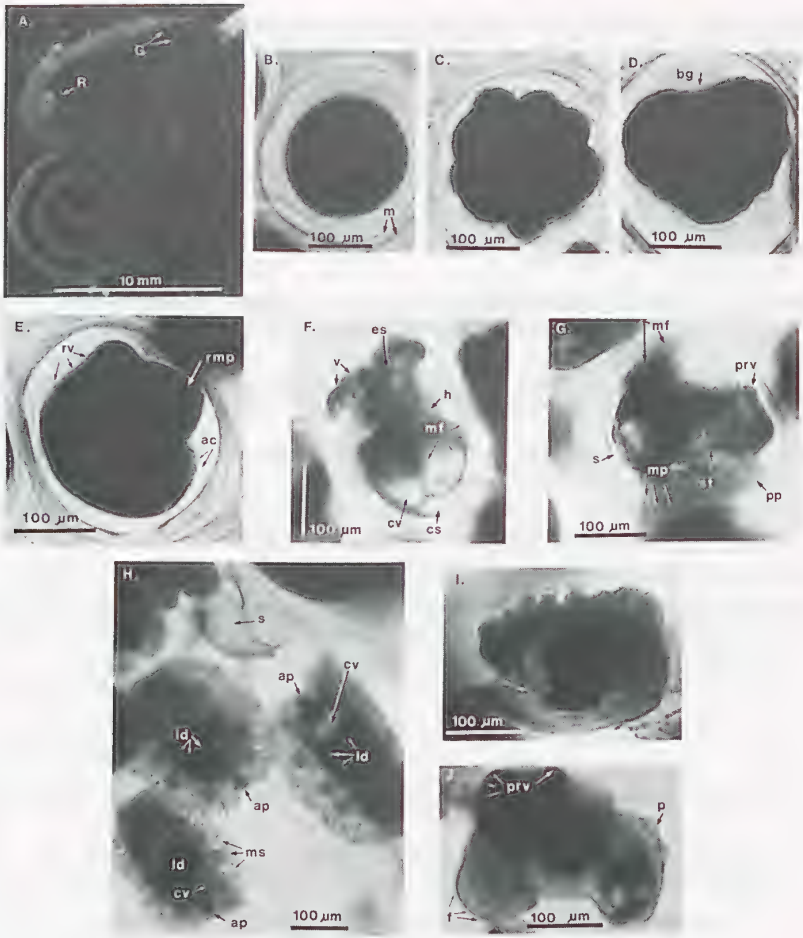

PLATE 1

Rostanga arbutus. A. Adult with egg mass; note rhinophores (R) and slightly retracted gill plume (G). B. One-cell stage 4h after oviposition and just before expulsion of polar bodies; note capsule consists of two membranes (m). C. Fourth cleavage 23h after oviposition. D. Lateral view of 3-day old gastrula; note heart-shaped appearance and blastoporal groove (bg). E. Right-lateral view of early veliger 5.5 days old, showing anal cells (ac), rudimentary velum (rv) and rudimentary metapodium (rmp). F. Dorsal view of 11-day old late veliger, showing a partially jettisoned cap-shaped shell (cs), mantle fold (mf), larval heart (h), eye spots (es), velum (v) and clear vesicle (cv). G. Right lateral view of 11.5-day old late veliger, showing a partially resorbed velum (prv), statocysts (st), propodium (pp) and metapodium (mp), shell (s) and mantle fold with papillae (mf). H. Dorsal view of recently hatched juveniles, showing an anal complex (ap) behind a clear vesicle (cv) and left digestive diverticulum (ld); note spicules in mantle skirt (ms) and type 1 shell (s). I. Right lateral view of recently hatched juvenile, showing papillae with spicules. J. Left lateral view of a recently hatched abnormal juvenile cultured at 25°C/40‰S; note foot with short fine cilia (f), partially resorbed velum (prv) and dorsum with papillae (p). Juveniles which hatched when conditions varied had the same appearance as those cultured in 25°C/40‰S.

TABLE 1. *Rostanga arbutus.* Chronology of developmental stages from oviposition to hatching for embryos incubated at 20-21°C and 34‰S.

Time	Developmental stages
(hours)	
0	Oviposition
16-17	Third cleavage (3C)
23	Fourth cleavage (4C)
(days)	
1.3	Morula (M)
1.5	Blastula (B)
2.9	Gastrula (G)
4.5	Trochophore (T): occurrence of invagination and eversion of shell gland; appearance of rudimentary metapodium and velum
5.5	Early veliger (EV): formation of shell; appearance of anal cells; short cilia on prominent rudimentary velum; and stomodeal invagination
9	Middle veliger (MV): slowly rotating embryo with completely formed shell; a perivisceral cavity; rudimentary mouth; partially formed alimentary tract; a clear vesicle (secondary larval kidney) and a bilobed velum with short cilia
11	Late veliger (LV): shelled embryo with fully differentiated alimentary tract with two asymmetric digestive diverticula; two eye spots; statocysts; propodium and metapodium; appearance of larval heart
13	Juvenile (J): shelless vermiform juvenile with papillae on dorsum; mantle skirt with spicules; rhinophoral rudiments; rotating rapidly inside capsule
15	Hatching (H)
4-5	Expulsion of 2nd polar body (PB)
7-8	First cleavage (1C)
12	Second cleavage (2C)

TABLE 2. *Rostanga arbutus.* Results of three, two-factor analyses of variance which compare proportions of embryos from various temperature-salinity regimes that were at, or beyond, three developmental stages at specific times after oviposition. Times tested were based on the developmental rates of embryos reared at 20-21°C/33-35‰S.

SOURCE OF VARIATION	PROPORTION OF EMBRYOS AT OR BEYOND THREE DEVELOPMENTAL STAGES AND TIMES		
	1C/12th hour	G/4th day	EV/5th day
SALINITY	$F_{2, 23} = 25.96^{***}$	$F_{2, 11} = 66.71^{***}$	$F_{1, 7} = 9.75^{*}$
TEMPERATURE	$F_{3, 23} = 1.68^{n.s}$	$F_{1, 11} = 2.5^{n.s}$	$F_{1, 7} = 14.61^{*}$
INTERACTION	$F_{6, 23} = 3.15^{*}$	$F_{2, 11} = 5.83^{*}$	$F_{1, 7} = 0.07^{n.s}$

n.s. : not significant, $P > 0.05$
* : $P < 0.05$
*** : $P < 0.001$

TABLE 3. *Rostanga arbutus.* Control experiments showing the proportions of embryos that hatched on the 12th day when reared in three salinities at 23°C. Dash indicates no hatching.

		Proportions Hatching	
Salinity S	*27*	*34*	*40*
	-	1.00	0.02
	-	0.98	0.04
	-	0.98	0.03
$\bar{x} \pm$ S.D.	*0*	*0.99 ± 0.01*	*0.03 ± 0.01*

TABLE 4. *Rostanga arbutus.* Non-optimal to optimal conditions: transfer experiments showing the proportions of embryos that hatched on the 14th day after oviposition, when embryos had been transferred at the 4C-M stage from three salinities kept at 13°C to 23°C/34‰S. Dash indicates no hatching.

		Proportions Hatching	
Salinity S	*27*	*34*	*40*
	-	0.16	0.08
	-	0.16	0.02
	-	0.18	-
$\bar{x} \pm$ S.D.	*0*	*0.17 ± 0.01*	*0.03 ± 0.04*

TABLE 5. *Rostanga arbutus.* Optimal to non-optimal conditions: transfer experiments showing the proportions of embryos that hatched on various days after oviposition, when embryos had been transferred at four embryonic stages from 23°C/34‰S to three salinities at 13°C. Dash indicates no hatching.

	Proportions Hatching *Stages of Transfer*			
Salinity S	*4C-M*	*G*	*EV*	*LV*
27	-	-	-	-
	-	-	-	-
	-	-	-	-
34	0.06	-	-	-
	0.02	-	-	-
	0.02	-	-	-
$\bar{x} \pm$ S.D.	0.03 ± 0.02	0	0	0
40	0.03	0.1	0.08	0.02
	0.08	-	0.04	0.04
	0.02	-	-	-
$\bar{x} \pm$ S.D.	0.04 ± 0.03	0.03 ± 0.06	0.04 ± 0.04	0.02 ± 0.02
Days to hatch:	53	44	42	29

FIGURE 1. *Rostanga arbutus.* The developmental rates of embryos reared in 34‰S at five temperatures. Each point plotted represents the mean of 50% or more embryos which were at or beyond a particular stage and time.

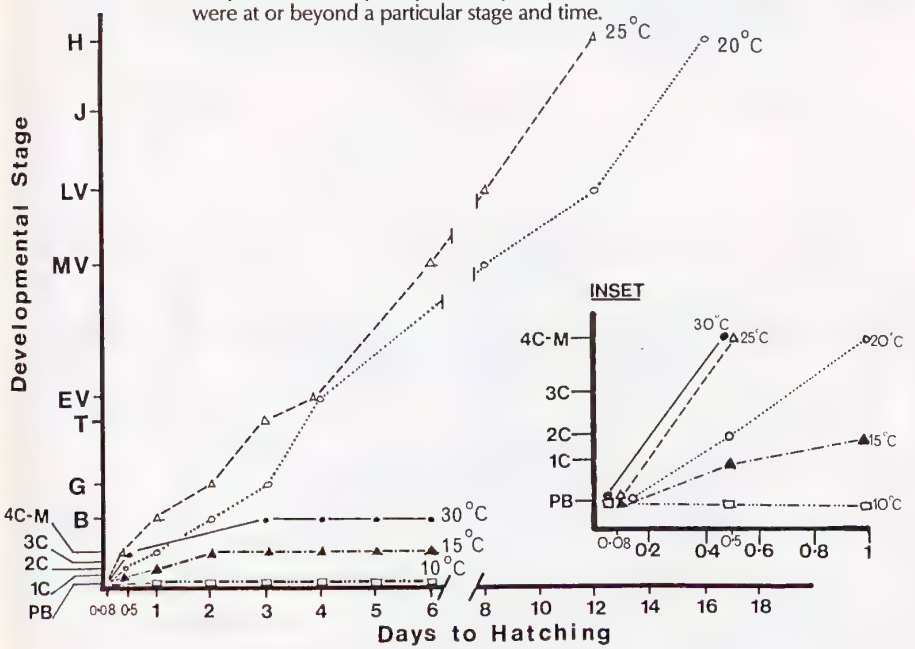

FIGURE 2. *Rostanga arbutus.* The developmental rates of embryos reared in 40‰ S at five temperatures. Except at hatching, each point plotted represents the mean of 50% or more embryos which were at or beyond a particular stage and time. At hatching each point plotted represents a mean of less than 3%.

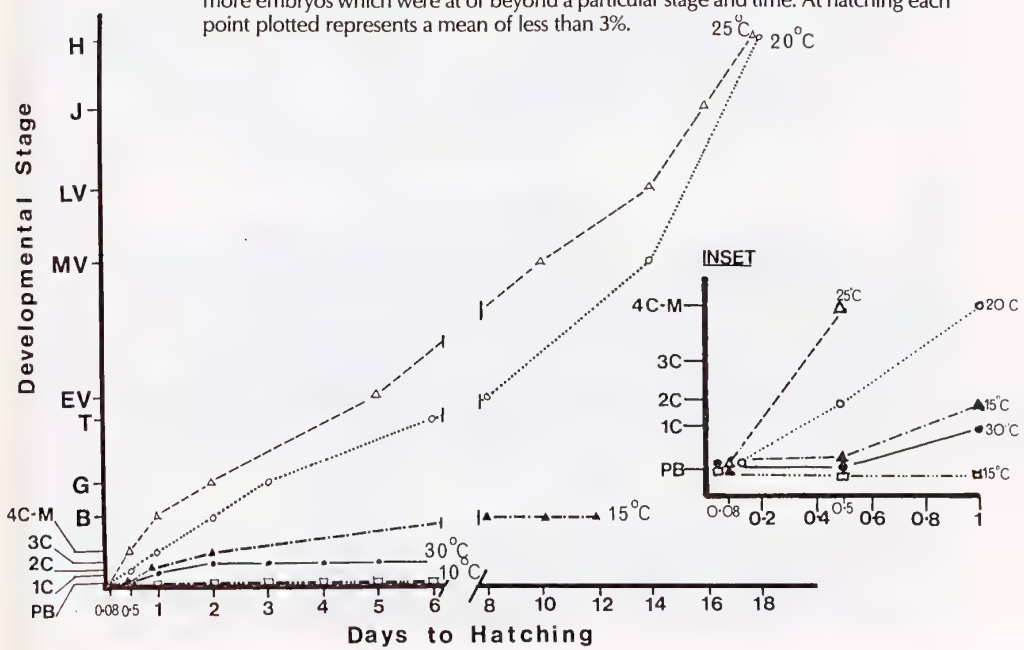

FIGURE 3. *Rostanga arbutus.* Figures below illustrate, for different temperatures, the total percentage of embryos which reached embryonic stages (clear histograms) and the percentage of this total which were abnormal (hatched histograms).